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Micro-wound detection on apple and pear fruit surfaces using sulfur dioxide

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Abstract

The fumigation of apple and pear fruit for 2 h with 2 ml/l of sulfur dioxide at room temperature enabled adequate detection of fresh and old micro-wounds varying from 0.1 to 5 mm in diameter. A bleached spot surrounded by a brown halo formed around the wounds allowing their visualization. Otherwise, the same symptoms were observed when fruit were treated for 2 h with 20 ml sodium metabisulfite solution at 20 g/l.

The incidence of wounding among SO_2 treated apples varied from 9 to 20% and from 13 to 24% in the 2001–2002 and 2002–2003 harvest periods, respectively. The susceptibility was higher on pear fruit which averaged 17–30% of wounded fruit at harvest for the same periods. Comparatively, the incidence of wounding varied between 5 and 11% when apple or pear fruit were evaluated visually for superficial defects. Additional wounds occurred during the sizing of 'Cameo' apples. Thus, the incidence of wounding was around 12 and 23% before and after sizing, respectively. SO_2 fumigation revealed an abraded surface two-fold larger on fruit after sizing resulting principally from impacts between adjacent fruit and between fruit and the bin sides.

Keywords: Wounds; Harvest; Sizing; SO_2; Sodium metabisulfite; Apples; Pears

1. Introduction

The extracellular cuticle protects plants against water loss (Schreiber and Reiderer, 1996) and invasion of microorganisms (Jenks et al., 1994; Markstadter et al., 2000). The breakdown of this protective layer is closely related to the firmness and plasticity of cell walls, and to the adhesive strength between neighboring cells (Banks, 1985). Breaking of cells generally results in openings that are required for the penetration of several pathogens. Consequently, numerous studies have been conducted to evaluate the risks of the level of surface injury that is observed at harvest and in moving fresh fruit from the tree to the packinghouse (Gordon, 1985; Sugar and Penwell, 1989; Spotts et al., 1998). The visualization of non-obvious wounds may be difficult to do. Thus, methods such as confocal laser scanning microscopy (CLSM) (Veraverbeke et al., 2001) and electrical impedance spectroscopy (Harker and Maindonald, 1994; Jackson and Harker,

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2000) were developed in order to determine the extent of tissue damage. Even though these methods are quite effective in detecting defects, their use on large samples remains difficult.

One of the most detrimental changes induced by wounding (cutting, cracking, breaking) is the induction of phenylpropanoid metabolism that results in the accumulation of phenolic compounds and subsequent tissue browning. In the case of injuries, the apple phenolics, localized mainly in cortex and skin (Robards et al., 1999) may be rapidly oxidized by polyphenol oxidase enzymes. Among several compounds, bisulfites are effective in preventing browning. Additionally, sulfur dioxide, in its various forms, is added to food to inhibit and control the growth of microorganisms, to inhibit enzyme-catalysed reactions, and to inhibit non-enzymic browning (Nelson and Ahmedullah, 1976; Li et al., 1999; Jiang et al., 2002). It has been shown that SO_2 applied at 0.5% may diffuse through natural openings and cause a bleached spot surrounded by a circular brown halo around the open lenticels on apples (Bompeix, 1972).

Invisible wounds (diameter less than 100 μm) cannot be detected visually and wide wounds are often difficult to estimate by visual observations. The aim of the present study was to assess the usefulness of SO_2 and sodium metabisulfite in wound detection. The capacity of SO_2 to hasten the browning of the wounded tissues was used to evaluate the extent of wounding on apple and pear fruit surfaces in practical conditions at harvest and during sizing.

2. Materials and methods

2.1. Plant material and wounding procedure

Freshly harvested ‘Golden Delicious’ apples and ‘Angelys’ pear fruit were wounded before being fumigated with SO_2. Each fruit was wounded at four equidistant locations in the equatorial area. Several combinations of diameter (0.1, 0.2, 0.4, 1.0, 5.0, 10 mm) and depth (1, 2, 4, 8 mm) of wounds were tested. Wounds with a diameter less than 1.0 mm were made with needles of corresponding diameter on which was mounted a wood block to control the wound depth. Wounds with a diameter more than 1.0 mm were made with a calibrated pinking-iron equipped with a sliding ring to control the depth. Five fruit were used in each combination and the experiment was repeated at least three times.

Table 1
Summary of SO_2 and sodium metabisulfite treatments applied to apple and pear fruit

	Time contact (min)				
	30	60	120	240	480
SO_2 doses (ml/l)					
0.5	X	X	X	X	X
1.0	X	X	X	X	X
1.5	X	X	X	X	X
2.0	X	X	X	X	
4.0	X	X	X		
Concentration of sodium metabisulfite (g/l)[a]					
5	X	X	X		
10	X	X	X		
20	X	X	X		

X indicates the effective combination.

[a] Sodium metabisulfite solution was applied at four surfaces of contact (10, 15, 20 and 25 cm^2/l) for each time and each concentration.

2.2. Sulfur dioxide treatment

The wounded apples and pears were SO_2-fumigated at different doses/time combinations as shown in Table 1. Fruit were placed in the upper part of an airtight glass container (40 l) (Fig. 1). In the bottom of the container was placed a mini-fan (Holmes, China) to allow a better gas distribution. Purified compressed sulfur dioxide (ESSECO-SPA, Italy) was expanded in a 60 ml syringe (Prolabo, France) and injected at atmospheric pressure through a port immediately sealed by

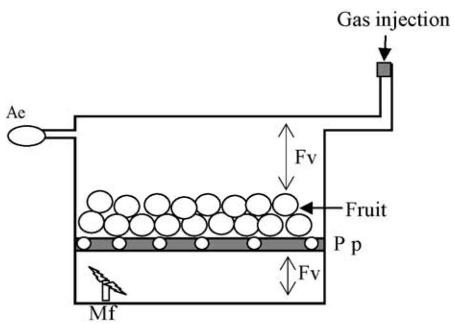

Fig. 1. The SO_2 and sodium metabisulfite treatment system (Fv: free volume, Ae: aerotonometer, Mf: mini-fan, Pp: porous patch).

a stop cock. An aerotonometer was connected to the container to check the stability of pressure during the treatment. The volume of gas to be injected was calculated in ml/l of free volume within the container after subtraction of the fruit volume (estimated by weight measurement). The treatment was conducted at 24 and 5 °C. After their fumigation, fruit were aerated for 24 h and the SO_2 diffusion (spot) was measured using slide calipers. Each SO_2 dose/time combination was replicated at least three times.

2.3. Effect of delay in storage after wounding on wound detection with SO_2

‘Golden Delicious’ apple fruit were wounded at two locations in the equatorial-area of each fruit. Wounds (1 and 2 mm wide and 2 mm deep) were made with calibrated needles. Fruit were separated in two batches and placed in plastic boxes. The first batch was stored at 20 °C and the second at 5 °C. At 0, 24, 48, 96, 192, 240 h after wounding, fruit were fumigated with compressed SO_2 (2 ml/l) for 2 h and than aerated for 24 h to measure the diameter of necrosis (SO_2 diffusion). Five fruit were treated at each time/temperature combination, and each combination was replicated at least two times.

To assess the diffusion of SO_2 through tissues, small pieces (4 cm square and 2 cm thick) which included the wound were removed around the wound area from ‘Golden Delicious’ apples which were previously SO_2 fumigated (2 ml/l). The pieces were dipped in 1% red tetrazolium (2,3,5-triphenyl tetrazolium chloride, Sigma T-8877) solution (pH 7.0) overnight at 30 °C.

2.4. Wound detection using sodium metabisulfite

‘Golden Delicious’ apple fruit were wounded as above (same diameters except 10 mm and same depths) and treated in the same system as with the compressed SO_2 (Fig. 1). The fruit were treated with sodium metabisulfite at different concentrations/surface contact combinations. A volume (20 ml) of the solution was poured into glass dishes (with the corresponding surface contact cm^2/l) and placed within the container. A summary of different treatments is given in Table 1. Each treatment (dose × time) was combined with four surfaces of contact: 10, 15, 20 and 25 cm^2/l (free volume). Five fruit were used in each combination (dose/time/surface contact), and each combination was repeated at least three times.

2.5. Extent of wounds on apples and pears at harvest

In the 2001–2002 and 2002–2003 harvest periods, three cultivars of apples, (‘Boskoop’, ‘Canada gris’, and ‘Elstar’), as well as ‘Conférence’ pears, all from Picardie (eastern France), and ‘Elstar’, ‘Braeburn’, and ‘Pirouette’ apples and ‘Williams’ and ‘Angelys’ pears from Val de Loire (western France) were surveyed for wounds. For each cultivar in each orchard, 250 fruit were harvested at random by several pickers and transported under typical conditions to the packinghouses where SO_2 fumigation was carried out. Fruit were placed in a plastic container (1 m^3) with two mini-fans to insure a better gas distribution. Twenty artificially wounded fruit (two wounds (1 mm wide) per fruit) of the same cultivars were placed in the bin, which was hermetically sealed by a polyethylene sheet. The fruit were fumigated with compressed SO_2 (2 ml/l) and held for 2 h at room temperature, after which they were aerated for 24 h before being inspected for wounds. An apple was counted as wounded if at least one wound was detected on the fruit. As controls, 100 fruit of each cultivar were evaluated visually for superficial wounds.

2.6. Extent of wounds on apples during sizing

In the 2003 packing period, three lots of ‘Cameo’ apples were surveyed for wounds during sizing. One hundred fruit (size 75–80, sized manually) were sampled at random from a cold room after 6 months of storage in a controlled atmosphere (1% O_2 and 2% CO_2). Following the sizing, another lot (100 fruit) was sampled after the fruit had been placed back in the bins. The SO_2 test (2 ml/l for 2 h) was carried out in the same manner as at harvest. This survey of fruit was repeated three times during the same season.

2.7. Statistics

Data were analyzed with Minitab statistical software (version 13.2). Variance analysis was conducted for each trial and means separated at $P = 0.05$ using Student’s *t*-test when significant differences were observed.

3. Results

3.1. Artificial wound detection using SO_2 fumigation

Fumigation of wounded apples or pears with a sufficient SO_2 dose causes a bleached necrosis (spot) surrounded by a brown halo immediately around the necrotic area (Fig. 2). The capacity of SO_2 to detect and show wounds is expressed as the diffusion (mm) of the gas from the edge of the wound (Table 2). When SO_2 was applied at concentrations less than 1 ml/l, no wounds were detected. A dose of 1.5 ml/l allowed only the detection of wounds with a diameter more than 1 mm independently of the duration of treatment (Table 2). The optimal SO_2 concentration for detecting wounds independently of their diameter seems to be around 2 ml/l when applied for 2 h (Table 2). At a dose of 4 ml/l, when the time contact exceeded 60 min, SO_2 diffused over the entire fruit surface, preventing wound visualization. When fumigation was carried out at 5 °C, no necrosis was observed around the wound even though the treatment was extended. It should be noted that SO_2 fumigation induces the same patterns on pears (unpublished data) as discussed here on apple fruit.

The diffusion of SO_2 was similar on wounds that differed in depth. In contrast, the diffusion was positively related with the wound diameter when the latter did not exceed 5 mm, and remained unchanged for larger diameters (Fig. 3). These observations were reinforced by the cell viability test. Only cells forming the necrosis and those situated in the upper layer of the palisade parenchyma remained uncolored when dipped in 1% red tetrazolium, while the cells underneath and those situated beyond the necrosis area turned pinkish-red, proving their viability.

Consequently, the combination 2 ml/l of SO_2 applied for 2 h was selected for the following tests.

3.2. Effect of delay in storage after wounding on wound detection by SO_2

SO_2 was able to detect wounds independently of the period between wounding and fumigation. The SO_2 diffusion declined when apples were stored an addi-

Table 2
SO_2 diffusion distance from the wound border on 'Golden Delicious' fruit after fumigation at 20 °C

SO_2 dose (ml/l)	Time of contact (min)	SO_2 diffusion (mm)[a] (±S.E.)				
		Wound diameter (mm)				
		0.1	0.2	0.4	1.0	5.0[c]
1	30	0.0	0.0	0.0	0.0	0.0
	60	0.0	0.0	0.0	0.0	0.0
	120	0.0	0.0	0.0	0.0	0.9a ± 0.2
	240	0.0	0.0	0.0	0.3a ± 0.1	1.3a ± 0.7
	480[b]	0.0	0.0	0.3	1.3b ± 0.3	2.1b ± 0.6
1.5	30	0.0	0.0	0.0	1.2b ± 0.6	2.2b ± 0.2
	60	0.0	0.0	0.0	2.3c ± 0.5	2.3b ± 0.5
	120	0.0	0.0	0.0	2.6c ± 0.6	2.6b ± 0.4
	240[b]	0.0	0.0	0.0	2.6c ± 0.6	2.6b ± 0.4
2.0	30	0.0	0.0	1.9a ± 0.2	3.0c ± 0.6	5.0c ± 1.1
	60	0.0	0.0	2.0a ± 0.2	4.0d ± 0.9	6.0d ± 0.8
	120	1.0a ± 0.1	2.5a ± 0.4	3.5b ± 0.8	4.6d ± 0.9	6.0d ± 0.8
4.0	30	0.7a ± 0.1	1.1b ± 0.1	1.9a ± 0.4	3.4c ± 0.8	6.3d ± 1.9
	60	1.7b ± 0.3	2.4a ± 0.7	3.8b ± 1.1	5.1d ± 1.5	6.5d ± 1.4

Data with different letters are significantly different at $P = 0.05$ according to Student's *t*-test. S.E. indicates standard error.

[a] SO_2 diffusion was measured between the wound border and the brown halo. The depth of wounds was 2 mm (same observations were done with wounds of 1, 4 and 8 mm in depth).

[b] Data of apples treated for 240 min with SO_2 doses more than 2 ml/l and for 480 min for doses over 1 ml/l are not shown.

[c] Data of wounds 10 mm in diameter are roughly similar to those of 5 mm. No wounds were detected when fruit were treated at 5 °C.

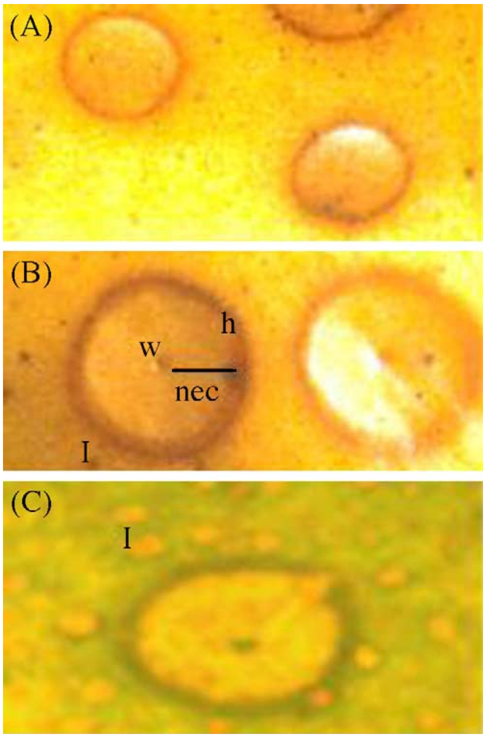

Fig. 2. Wound detection on apples and pears using SO_2 fumigation at 2 ml/l (free volume) for 2 h at 20 °C. (A) and (B), are wounds 0.1 and 1 mm wide, respectively detected on 'Golden Delicious' apples. (C) shows a wound 2 mm wide detected on 'Angelys' pears. (w: wound, l: lenticel, h: halo, nec: necrosis).

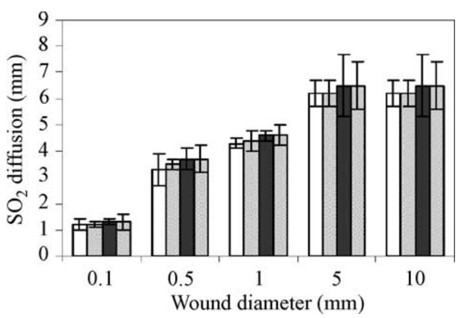

Fig. 3. Effect of wound diameter and wound depth on the capacity of wound detection by SO_2. Wounds of different diameter and depth (□) 1 mm, (▨) 2 mm, (■) 4 mm, (■) 8 mm were made on 'Golden Delicious' apples which were treated with 2 ml/l of SO_2 for 2 h at 20 °C. The values were measured after 24 h of fruit aeration following the SO_2 fumigation. Points are means of 30 evaluations and bars represent standard deviations.

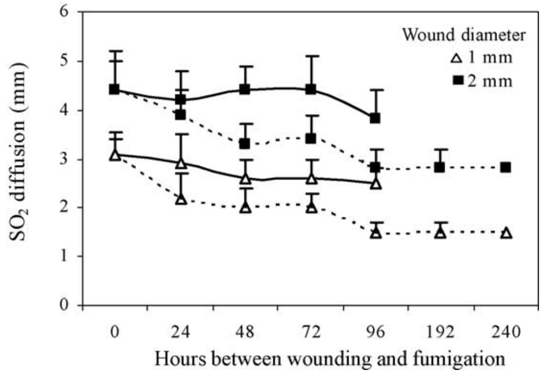

Fig. 4. Effect of delay in storage after wounding on the capacity of wound detection by SO_2 fumigation. 'Golden Delicious' apples were wounded and held at 5 °C (—) and at 20 °C (- - -) for selected times before fumigation (2 ml/l of SO_2 for 2 h at 20 °C). Points are means of 20 evaluations and bars represent standard deviations.

tional 96 h at 5 °C instead of 20 °C. Thus, diffusion of the gas with wounds 1 mm wide declined from 3 mm on freshly inflicted wounds to 1.5 mm when the wounded fruit were stored an additional 96 h at 5 °C before fumigation (Fig. 4). After 96 h, the diffusion of SO_2 remained unchanged. At 5 °C, wounds 2 mm in diameter showed a comparable trend in the reduction in diffusion after SO_2 fumigation. When the wounded fruit were stored for an additional 96 h at 20 °C, wounds 1 and 2 mm wide produced necroses of 2.9 and 4 mm in diameter, respectively (Fig. 4).

3.3. Wound detection using sodium metabisulfite

Both concentration of sodium metabisulfite and its surface contact with the free volume within the container should be considered when this compound is used for wound detection. Thus, wounds were not detected with 5 g/l sodium metabisulfite as at 10 g/l combined with a surface contact of 10 cm^2/l. The later dose applied at 15 cm^2/l detected only wounds with a diameter greater than 0.2 mm, and 20 cm^2/l is needed for wounds 0.1 mm wide (Table 3). At 20 g/l, sodium metabisulfite should be applied with a surface contact of more than 15 cm^2/l to detect wounds independently of their diameter (Table 3). All concentrations combined with all surfaces of contact were unable to detect wounds when the duration of treatment was less than 60 min. When the fumigation was applied for 60 min, only the higher dose (20 g/l) combined with the max-

Table 3
SO_2 diffusion distance from the wound border on 'Golden Delicious' fruit after fumigation using sodium metabisulfite at 20 °C

Sodium metabisulfite concentration (g/l)	Contact surface (cm^2/l)	SO_2 diffusion (mm)[a] (±S.E.)				
		Wound diameter (mm)				
		0.1	0.2	0.4	1.0	5.0
10	10	0.0	0.0	0.0	0.0	0.0
	15	0.0	1.0a ± 0.5	1.4a ± 0.6	1.8a ± 0.4	2.2a ± 0.4
	20	0.6a ± 0.3	1.4a ± 0.3	2.0b ± 0.7	2.4b ± 0.5	2.6a ± 0.6
	25	1.1b ± 0.4	2.1b ± 0.2	2.4b ± 0.8	2.6bc ± 0.4	3.2bc ± 0.7
20	10	0.0	0.0	0.0	0.0	0.0
	15	0.9a ± 0.4	1.6a ± 0.2	1.9b ± 0.3	2.2b ± 0.4	2.6a ± 0.8
	20	1.2b ± 0.8	1.9b ± 0.4	2.4b ± 0.4	2.7bc ± 0.4	3.0b ± 0.7
	25	1.4b ± 0.4	2.4b ± 0.7	2.7c ± 0.5	3.0c ± 1.2	3.6c ± 1.1

Wounds were 2 mm in depth. Data are means of 30 evaluations and S.E. indicates standard error. Data with different letters are significantly different at $P = 0.05$ according to Student's *t*-test.
[a] SO_2 diffusion was measured between the wound border and the brown halo after 2 h of fumigation at 20 °C.

imal surface contact (25 cm^2/l) detected the wounds (unpublished data).

3.4. Survey of fruit for wounds at harvest

When fruit were surveyed for wounds using SO_2 fumigation, the incidence of wounding in apples varied from 9 to 20% and from 13 to 24% in the 2001–2002 and 2002–2003 harvest periods, respectively. For the same periods, the incidence of wounding among pears varied from 17 to 23% and 20 to 30%, respectively (Table 4). Globally, the majority (80%) of wounded apples and pears showed one wound on their surface. 'Pirouette' and 'Elstar' apples were more susceptible to damage. 'Conférence' pears harvested in Picardie where more susceptible than 'Angelys' pears in Val de Loire (Table 4). The percentage of wounds revealed on the visually rated fruit was one- to two-fold lower than on the SO_2 treated fruit. Incidence of wounding detected visually varied from 5.3 to 10.3% in apples and between 7.9 and 12% among cultivars of pear fruit (Table 4).

The largest wound diameter revealed by the SO_2 fumigation was recorded in 'Pirouette' apples, 1.6 mm,

Table 4
Incidence of wounding on apple and pear fruit at harvest and wound diameter revealed by SO_2

Area	Cultivar	Wounded fruit (%)[a]					
		2001–2002		2002–2003		Wound diameter[b] (±S.E.)	
		Control	SO_2 treated	Control	SO_2 treated	2001–2002	2002–2003
Picardie	Canada gris	6.0	13	9.0	20	1.0 ± 0.6	1.4 ± 0.7
	Boskoop	5.3	9	8.2	13	1.2 ± 0.3	1.3 ± 0.4
	Elstar	7.1	12	10.3	18	1.1 ± 0.5	1.4 ± 0.6
	Conférence (pear)	10.2	23	12.0	30	1.5 ± 0.4	1.8 ± 0.5
Val de Loire	Elstar	9.6	15	10.1	16	1.4 ± 0.4	1.6 ± 0.5
	Braeburn	7.9	10	7.1	13	1.5 ± 0.2	1.2 ± 0.3
	Pirouette	10.1	21	10.2	24	1.6 ± 0.8	2.0 ± 0.9
	Williams (pear)	9.9	26	12.0	28	1.5 ± 0.6	2.1 ± 0.7
	Angelys (pear)	8.8	17	7.9	20	1.5 ± 0.2	1.6 ± 0.3

[a] Percentage of wounded fruit was assessed by SO_2 fumigation (2 ml/l for 2 h) on 250 fruit for each cultivar and on 100 fruit for the control (assessed visually).
[b] Data are means of wounded diameter on the SO_2 treated fruit, wounds with diameter more than 2.5 mm are not included. S.E. indicates standard error.

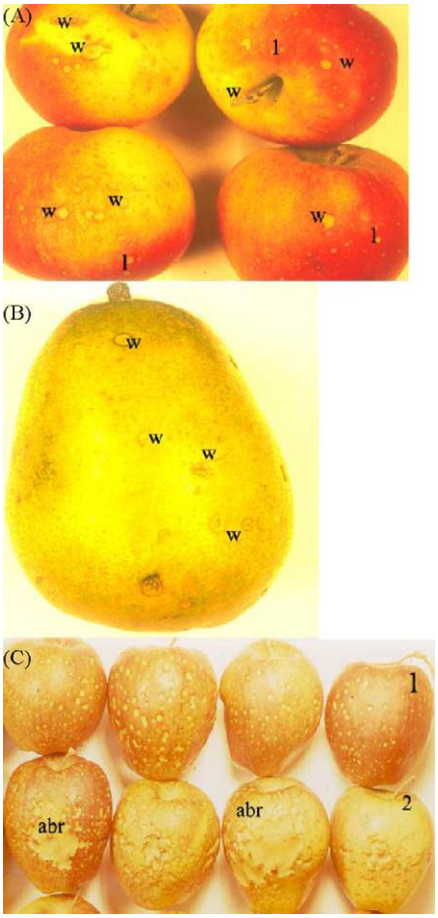

Fig. 5. Natural wounds on 'Braeburn' apples (A) and 'Angelys' pears (B) detected at harvest by SO_2 fumigation. (C) shows the width of the abraded area on 'Cameo' apples sampled from the bin after sizing (2) compared to the control (manually sized) (1). Fruit were fumigated at 20 °C with 2 ml/l of SO_2 for 2 h and aerated for 24 h before being accessed (w: wound, l: lenticel, Abr: abrasion).

and the smallest, 1 mm in 'Canada Gris' apples. On pears, average wound size varied from 1.5 mm in 'Angelys' and 2.1 mm in 'Williams' (Table 4). Generally, apples were more susceptible to injuries in the Val de Loire area compared to those harvested in Picardie. Some symptoms of natural wounds detected using SO_2 at harvest are shown in Fig. 5A and B.

3.5. Survey of fruit for wounds during sizing

After 6 months of storage, additional wounds occurred during the sizing of 'Cameo' apples. The SO_2 fumigation revealed that the extent of wounding was 12.1 and 23%, respectively before and after sizing. In comparison, visual evaluation showed that the percentage of wounding increased from 8.1% before sizing to 10.6% after sizing (Table 5). The most important fact at this phase was the amount of bruising that was revealed by SO_2 on fruit (Fig. 5C). Thus, after sizing, 28% of 'Cameo' apples showed an abraded surface larger than 5 cm^2, against only 6% before sizing (Table 5).

Table 5
Wounded fruit (%) and percentage of bruised fruit on 'Cameo' apples during sizing

Treatment	Wounded fruit (%)[a]		Bruised fruit (%)[b]	
	Control	SO_2 treated	$S < 5\ cm^2$	$5\ cm^2 < S < 20\ cm^2$
Before sizing	8.1a	12.1a	8	6
After sizing	10.6a	23.0b	24	28

S: surface of the bruised area; data are the means of evaluations on 50 fruit at each step.
[a] Means of three replications during the 2003 packing season. Fruit were treated with SO_2 (2 ml/l) for 2 h.
[b] Number of bruised fruit recorded on 50 fruit at each sample.

4. Discussion

Avoidance of injuries during harvesting and handling is usually considered as an important disease reduction measure. Even though they are laborious to do, especially on large samples, visual or microscopic observations are generally used to quantify the phenomena.

The principal aim of this study was to develop a simple and useful test that allows a rapid and easier wound detection on apple and pear fruit. The fumigation of those fruit with a sufficient dose of SO_2 causes a bleached necrosis (spot) surrounded by a circular brown halo around wounds and lenticels. The brown halo results from the oxidation and polymerization of phenolic compounds, including the red anthocyanins, by polyphenol oxidase (Zauberman et al., 1989; Underhill and Critchley, 1992). The optimal SO_2 dose that allows a better wound detection is 2 ml/l (free volume). At weak doses, SO_2 may be neutralized after its combination with several components of apples tissues such as pyruvic acid, 2-oxoglutaric acid and

acetaldehyde (Rousseau, 2001). This explains the fact that SO_2 was unable to mark wounds when applied at low doses. When its concentration exceeds 4 ml/l, the gas causes a severe bleaching on the fruit surface that prevents wound detection. The capacity of SO_2 to show the wounds changes mainly depending on the wound diameter. Sulfur dioxide diffuses laterally through the gas spaces of apple tissue and causes cell death that forms the necrotic bleached area. Evidence for the mode of action of SO_2 indicates that its accumulation in the cytoplasm causes a disruptive decline in pH (Stratford and Rose, 1986). On the other hand, the resistance of skin to gas diffusion, associated with respiration of internal tissues, may result in their O_2 contents being lower than in the superficial cells (Cameron and Reid, 1982; Banks, 1985). Since oxygen is necessary for phenolic oxidation by PPO, its deficiency in the deep cells may inhibit tissue browning at these sites. Wounds were not detected when the fruit were fumigated at 5 °C. This supposes that at low temperatures, PPO activity may be inhibited. Furthermore, abscisic acid (ABA) has been shown to be involved in tissue protection by decreasing the visible surface injury at a very low SO_2 concentration, especially at low temperatures (Krizek et al., 1986, 2001).

The monitoring of such a test as developed here can be made more easier by the use of sodium metabisulfite solution at 10 g/l applied so as to ensure a surface contact of 20 cm^2/l (free volume). At these conditions, this compound generates sufficient doses of SO_2 that diffuses and shows up the injured tissues.

Freshly inflicted wounds are well detected by SO_2 fumigation, but also when fruit were held for an additional storage period after wounding. When the wounded fruit were stored at 5 °C, the diameter of necrosis decreased when the fumigation was applied 96 h after wounding. This reinforces previous observations which claimed that wound healing may occur in wounds on apples after an additional storage period of 4 days at 5 °C (Lakshiminarayana et al., 1987). However, the healing seems to be partial since some SO_2 diffused and marked the wounded tissues.

The SO_2 fumigation applied on apple and pear fruit at harvest revealed that the proportion of invisible wounds may vary from 10 to 50% of the total number of wounds. Only a small number of those wounds may be completely filled with cork tissue and impenetrable for microorganisms during storage. The other wounds remain susceptible to attack by pathogens. Incidence of wounding varied by cultivar and from year to year. Fruit should be harvested at the same level of maturity. Thus, late-picked fruit may be more susceptible to physical accidents and decay (Kupferman, 1993). Furthermore, less care on the part of the workers harvesting the fruit might also create additional wounds. During fruit transfer from the tree to the packinghouse, the quality of roads, and distance and speed variation may influence the extent of physical damage (Gordon, 1985). Visual evaluation has shown that the incidence of wounding was 4.3% in ‘Bosc’ pears harvested by workers paid by the hour, and averaged 14% when workers were paid by the number of bins harvested (Spotts et al., 1998). Finally, special care should be taken during sizing, since the fruit are in contact with hard surfaces. It was shown here that fruit could be highly abraded after sizing, increasing by this their susceptibility to several pathogens that occur in packinghouses.

In conclusion, this new method based on the use of sulfur dioxide in its various forms not only enables wound detection but also leads to quantification of this phenomenon on representative samples of apple and pear fruit, and can be used on numerous fruits and vegetables. When applied at the concentration as shown here, sulfur dioxide remains harmless and can be used in practical conditions, such as in packinghouses. It could lead to the development of an approach that may alleviate such problems in order to improve the quality of harvesting and handling procedures. This test may also help in the study of infection pathways to understand the parasitic cycle of some fungi which require a wound to penetrate and infect the plant.

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References

Banks, N.H., 1985. Estimating skin resistance to gas diffusion in apples and potatoes. J. Exp. Bot. 36, 1842–1850.

Bompeix, G., 1972. Perméabilité lenticellaire des pommes et susceptibilité aux attaques fongiques. Bull. Soc. Bot. Fr. (Acta Botanica Gallica) 115, 75–81.

Cameron, A.C., Reid, M.S., 1982. Diffusive resistance: importance and measurement in controlled atmosphere storage. In: Richardson, D.G., Meheriuk, M. (Eds.), Controlled Atmosphere for Storage and Transport of Perishable Agricultural Commodities. Oregon State University School of Agriculture, Symposium Series No. 1, Timber Press, Beaverton, Oregon, pp. 171–180.

Gordon, F.M., 1985. Reducing handling injuries during fruit harvest. Postharvest Pomol. Newslett. 3, 11–15.

Harker, F.R., Maindonald, J.H., 1994. Ripening of nectarine fruit, changes in the cell wall, vacuole, and membranes detected using electrical impedance measurements. Plant Physiol. 106, 165–171.

Jackson, P.J., Harker, F.R., 2000. Apple bruise detection by electrical impedance measurement. HortScience 35, 104–107.

Jenks, M.A., Joly, R.J., Peters, P.J., Rich, P.J., Axtell, J.D., Ashworth, E.N., 1994. Chemically induced cuticle mutation affecting epidermal conductance to water vapour and disease susceptibility in *Sorghum bicolor* (L). Moench. Plant Physiol. 105, 1239–1245.

Jiang, Y., Zhang, Z., Joyce, D.C., Ketsa, S., 2002. Postharvest biology and handling of longan fruit (*Dimoacarpus longan* Lour.). Postharvest Biol. Technol. 26, 241–252.

Krizek, D.T., Mirecki, R.M., Semeniuk, P., 1986. Influence of soil moisture stress and abscisic acid pre-treatment in modifying SO_2 sensitivity in poinsettia. J. Am. Soc. Hort. Sci. 111, 446–450.

Krizek, D.T., Terry, P.A., Upadhyaya, A., Caldwell, C.R., Mirecki, R., 2001. Changes in abscisic acid, stomatal conductance, and antioxidants during low temperature preconditioning against SO_2 injury in contrasting cultivars of coleus. Biotronics, 30.

Kupferman, E., 1993. Preharvest practices to minimize postharvest decay. Tree Fruit Postharvest J. 4, 7–8.

Lakshiminarayana, S., Sommer, N.F., Polito, V., Fortlage, R.J., 1987. Development of resistance to infection by *Botrytis cinerea* and *Penicillium expansum* in wounds of mature apple fruits. Phytopathology 77, 1674–1678.

Li, X.P., Pang, X.Q., Zhang, Z.Q., Ji, Z.L., Li, T., 1999. Effects of SO_2 on cold storage and shelf-life of longan fruits. J. South China Agr. Univ. 20, 77–80.

Markstadter, C., Federle, W., Jetter, R., Riederer, M., Holldobler, B., 2000. Chemical composition of the slippery epicuticular wax blooms on Macaranga (*Euphorbiaceae*) ant-plants. Chemoecology 10, 33–40.

Nelson, K.F., Ahmedullah, H., 1976. Packaging and decay-control for storage and transit of table grapes for export. Am. J. Enol. Viticult. 27, 74–79.

Robards, K., Prenzler, P.D., Tucker, G., Swatsitang, P., Glover, W., 1999. Phenolic compounds and their role in oxidative processes in fruits. Food Chem. 66, 401–436.

Rousseau, S., 2001. Importance de l'eau libre sur le développement des micro-organismes. Application à l'étude de la sur-maturation du raisin provoquée par *Botrytis cinerea*, Thesis. Université Bordeaux 2, France.

Stratford, M.A., Rose, H., 1986. Transport of sulfur dioxide by *Saccharomyces cerevisiae*. J. Gen. Microbiol. 132, 1–6.

Schreiber, L., Reiderer, M., 1996. Determination of diffusion coefficients of octadecanoic acid in isolated cuticular waxes and their relationship to cuticular water permeabilities. Plant Cell Environ. 19, 1075–1082.

Spotts, R.A., Sanderson, P.G., Lennox, C.L., Sugar, D., Cervantes, L.A., 1998. Wounding, wound healing and staining of mature pear fruit. Postharvest Biol. Technol. 13, 27–36.

Sugar, D., Penwell, M., 1989. Packing 'Bosc' pears. In: Proceedings of the Fifth Ann Warehouse Seminar, vol. 28, Wash. State Hort. Assoc., Marsh, Wenatchee, USA, p. 29.

Underhill, S.J.R., Critchley, C., 1992. The physiology and anatomy of lychee (*Litchi chinensis* Sonn.) pericarp during fruit development. J. Hort. Sci. 67, 437–444.

Veraverbeke, E.A., Van Bruaene, N., Van Oostveldt, P., Nicolaï, B.M., 2001. Non destructive analysis of the wax layer of apple (*Malus domestica* Borkh.) by means of confocal laser scanning microscopy. Planta 213, 525–533.

Zauberman, G., Ronen, R., Akerman, M., Fuchs, Y., 1989. Low pH treatment protects litchi fruit colour. Acta Hort. 269, 309–314.